# Social instability increases plasma testosterone in a year-round territorial neotropical bird

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In many vertebrates, elevated levels of plasma testosterone (T) are important for reproduction and territorial aggression. However, many tropical birds reproduce and defend territories while plasma T-levels are basal. We studied how aggression and T-levels are regulated in male neotropical spotted antbirds, which defend territories year-round in the Panamanian rainforest. Although spotted antbirds reproduce seasonally, T-levels of individual males often remained at baseline ($0.2\,ng\,ml^{-1}$) throughout the year, even in courting males. On the other hand, T-levels were elevated (maximally to $1.57\,ng\,ml^{-1}$) during periods of social instability at any time of the year, even when males had entirely regressed gonads. Experimental territorial intrusions (broadcast of conspecific song) confirmed these observations by showing that T-levels increased after about two hours of playback time. Our data suggest that spotted antbirds avoided the potential costs associated with constantly high plasma T-levels (e.g. increased mortality rates). Contrary to temperate-zone birds, spotted antbirds had the potential to react to social challenges with an increase of plasma T year-round. These results are, to our knowledge, presently unique, but may apply to many vertebrate species that inhabit the tropics.

**Keywords:** testosterone; tropical bird; rainforest; territoriality; antbird; social challenge

## 1. INTRODUCTION

The steroid hormone testosterone (T) is ubiquitous in vertebrates and serves important organizational and activational roles in behaviour (e.g. Hadley 1992). One prominent role of T is its involvement in aggression, especially in the context of territoriality (for overviews, see Balthazart 1983; Wingfield et al. 1987, 1990; Moore & Marler 1988; Wingfield & Hahn 1994). Temperate-zone birds have high T-levels during the breeding season and especially during the courtship phase (Wingfield et al. 1987). Paradoxically, many tropical birds have only very low plasma T-levels, but are nevertheless highly aggressive and territorial (Levin & Wingfield 1992; Wingfield et al. 1991, 1992; Dittami & Gwinner 1990; Wikelski et al. 1999b). Furthermore, many tropical birds are territorial year-round despite the fact that they reproduce seasonally (Dittami & Gwinner 1990; Wikelski et al. 1999a). We wanted to know how year-round territoriality in birds is physiologically regulated, and especially if T is involved in this regulation.

We therefore decided to study hormonal changes during territoriality in male neotropical spotted antbirds (*Hylophylax n. naevioides*). Spotted antbirds offer the right combination of behavioural and physiological traits necessary to study the regulation of year-round territoriality: (i) they only breed within the rainy season (in Panama from April to October) but aggressively defend

territories throughout the year as socially monogamous pairs (Willis 1972; Sieving 1992; Hau et al. 1999a); (ii) despite a constant high level of territorial aggression, they only show very low levels of plasma T throughout the year (Wikelski et al. 1999b). Being facultative followers of army ants, spotted antbirds often forage on insects that are flushed off by raiding army ant swarms (Formicidae). Raiding ants are usually accompanied by a large number of taxonomically diverse antbirds (Formicariidae), including many spotted antbird individuals that may temporarily invade a conspecific territory (up to eight males, M. Wikelski, H. Hau and J. Nesbitt, personal observations; Willis 1972). Despite these conspecific intrusions and episodic invasions of neighbouring territories, spotted antbirds have relatively stable territorial neighbourhoods and individuals may remain territorial in the same area for at least 11 years (Willis 1972).

We can derive predictions about how year-round territoriality is regulated in tropical birds by expanding present models for the regulation of aggression in temperate-zone birds. Many of the latter are not territorial in winter and thus have baseline levels of plasma T at this time (e.g. Wingfield & Farner 1976; Wingfield et al. 1997). In spring, plasma T-levels rise about tenfold to the breeding levels that regulate the expression of reproductive accessory organs and some secondary sexual characters, sperm maturation, and that set the physiological stage for further rapid and high (again tenfold) increases in plasma T during the courtship season

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(Wingfield & Marler 1988; Wingfield *et al.* 1990). Peak concentrations of plasma T are important especially during the territorial establishment period to enhance persistence of aggression during fights (Wingfield & Moore 1987; Wingfield 1994*a*,*b*; Hunt *et al.* 1997). Birds with experimentally elevated T-levels win more fights and can enhance their access to females (Beletsky *et al.* 1989; Raouf *et al.* 1997; see also Tokarz 1995), but not always (Ramenofsky 1984).

Some temperate-zone birds are also aggressive and territorial outside of the breeding season when gonads are regressed and plasma T is low as, for example, in their wintering areas (Silverin 1980; Wingfield 1984*a*, 1994*b*; Silverin *et al.* 1989; Logan & Wingfield 1990; Schwabl 1992; Gwinner *et al.* 1994; Soma & Wingfield 1999). The fact that aggression enhances plasma T during the breeding season was accounted for by the 'challenge hypothesis' (Wingfield *et al.* 1990). According to this hypothesis, T enhances aggressive behaviour during times of social instability such as during the annual establishment of territories in spring. The proposed means by which T enhances aggression is through increased persistence during behavioural encounters (e.g. Wingfield 1994*a*,*b*). The challenge hypothesis makes no prediction about T and territoriality in autumn. However, the lack of plasma T during autumn–winter territoriality was interpreted as a protection against the unwanted detrimental effects of the hormone (Wingfield & Hahn 1994; Wingfield *et al.* 1997). Continuously high plasma T-levels may cause cancer, depress the immune system and parental behaviour, or lower the long-term survival probability of birds (Dufty 1989; Hegner & Wingfield 1987; Wingfield *et al.* 1997; Ketterson *et al.* 1992; Hillgarth *et al.* 1997). Because of these negative effects, organisms should have high plasma T-levels only when they help to boost fitness, which is the case, for example, during territorial disputes in breeding birds.

We extend the challenge hypothesis here to tropical birds and predict that year-round territorial tropical birds should have low plasma T in order to avoid the cost of constantly high hormone levels. However, individuals should be able to increase plasma T-levels— independently of other physiological pathways—during social challenges, in order to fight off territorial intruders. Such events may happen during any time of the year.

The data available at present do not allow an evaluation of the challenge hypothesis for tropical birds because hormone data in all other studies were only taken at the population level, i.e. not repeatedly on the same individuals. To advance our understanding, we urgently needed data on individual hormone changes in the context of social stability–instability throughout the year. We therefore investigated plasma T changes and territorial behaviour in individual spotted antbirds.

## 2. MATERIAL AND METHODS

We studied male spotted antbirds from December 1995 until October 1997 along Pipeline Road in Soberania National Park, a lowland 22 000 ha moist forest in the central Republic of Panama (9° N, 79° W). Spotted antbirds (Thamnophilidae) are small (17 g) suboscine passerines with a presumably purely neotropical phylogenetic history (Sibley & Monroe 1990). Birds

were caught in mist nets and were attracted to the nets with song playbacks broadcast using a Sony tape recorder with Sony active loudspeakers. Songs used in playback had been taped from several territorial males within the study plot at a distance of about 5 m. Territory maps were drawn by eye to include all locations where individual spotted antbirds were caught or observed as a pair (8–15 observations per individual bird). Territory sizes were between 3 and 4 ha (cf. Willis 1972). We broadcast songs in the presumed centre of a territory. The duration of playback time was recorded. Territorial spotted antbirds always responded to playback. Individuals could hear the playback throughout their territory, but presumably not beyond it, because spotted antbird songs do not carry much further than 100 m, i.e. not much outside their territories (M. Wikelski and M. Hau, personal observations; W. D. Robinson, personal communication).

### (a) *Sampling*

Upon capture, birds were banded, laparotomized (Hau *et al.* 1998), and a small (*ca.* 200 μl) blood sample was taken from the wing vein. Blood was collected in heparinized capillary tubes, stored on ice and centrifuged within 10 h. Plasma was collected and 10 μl β-propiolactone was added according to US-import regulations, thereby diluting samples by about 10%. Final calculations of T-concentrations corrected for this dilution. β-propiolactone destroys viruses, but is indifferent to assay procedures (cf. Wingfield & Lewis 1993; Hau *et al.* 1998). Plasma was then frozen at −20 °C and transported back to Seattle on dry ice.

### (b) *Radioimmunoassays*

The plasma levels of T were measured with an indirect radioimmunoassay after separation of hormones on a chromatography column (Wingfield & Farner 1975; Wingfield *et al.* 1991). Trace label (20 μl) was added to all samples to determine recovery values (73 ± 9%). Water blanks were taken through the entire assay procedure and were usually found to be below a detection limit. The accuracy of the hormone standards was 6.9%. Intra-assay variation was 1.7% (mean of four assays). Inter-assay variation was 4.6% (between $n = 6$ assays). Assay sensitivity was at $0.2 \pm 0.06$ ng ml$^{-1}$ (see Wingfield & Farner (1975) for methods). Whenever samples were below the detection limit (53% of 40 samples), they were set at 0.2 ng ml$^{-1}$ as the highest possible value. This provided a conservative estimate for statistical comparisons.

### (c) *Breeding seasons and behavioural observations*

To compare data between individuals and years, we categorized the reproductive state of birds into four stages. (i) The non-breeding season was defined as the time when all birds had small, non-functioning gonads (September to February). (ii) Pre-breeding period was the time when gonads increased in size but no breeding took place (up to half maximal gonad size, March to April). (iii) Courting period was the time when birds had half maximal to maximal gonad sizes, but no active nests were found or no birds were captured with active brood patches (both male and female develop brood patches in spotted antbirds; Willis 1972; M. Wikelski and M. Hau, personal observations). Birds were also classified as courting when they had lost clutches and prepared to re-nest (i.e. two weeks before replacement eggs were laid). (iv) The subsequent parental phase covered most of the wet season until gonads regressed again to half-maximal sizes in about August to September. The parental
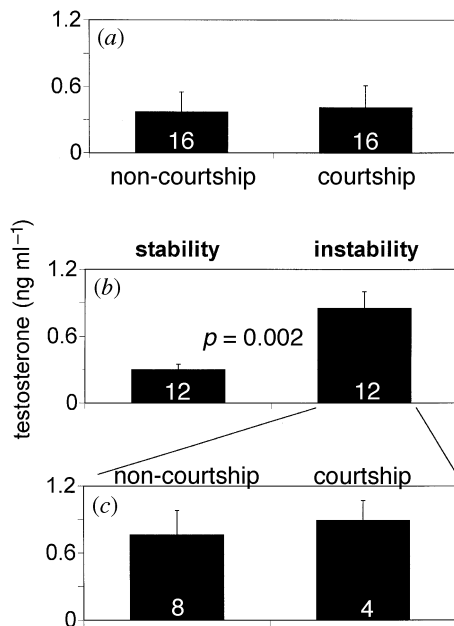
Figure 1. The maximum T-levels for individual males were similar during the courtship season and the non-courtship season (*a*). However, the maximal T-levels were significantly lower when individual males experienced social stability compared to times when those individuals experienced social instability (*b*). This was not due to the fact that T-levels for socially challenged males were higher during the breeding season (*c*).

phase included birds that were nesting, re-nesting and those that had fledglings.

We assumed that birds experienced periods of social instability whenever we captured three or more spotted antbirds in the territory of a known pair on the same day as the capture of the territory owner(s). We also included one case in our analysis where we happened to witness a territorial take-over. Owing to the dense understory foliage we were unable to determine exactly how long a territorial challenge lasted and how intense it had been. We do not know if more males than the ones captured interacted during a social challenge. Seven out of the 12 cases of social instability were positively associated with raids by army ants, while in the remainder of cases we could not exclude that army ants were in the area.

Data were processed with SPSS for Windows. Two-tailed test statistics were used. Data are given as means ± 95% confidence intervals except for regression equations (mean ± s.e.). Statistical significance for the results presented in figure 1*a*,*b* was accepted at the $\alpha = 0.025$ level, because the data set in figure 1*b* represents a subset of figure 1*a*. Statistical power at $\alpha_2 = 0.05$ was determined according to Cohen (1977).

## 3. RESULTS

An individual's maximum T-level during courtship did not differ significantly from its maximum T-level during any other season (Wilcoxon matched-pairs test, $n = 16$, $\mathcal{Z} = -0.11$, $p = 0.91$; figure 1*a*; only the maximum measured T-level of each male during each stage of the annual cycle was used to avoid pseudoreplication). The power of this test was $r > 0.995$. This result was not affected by the detectability of T in the assay procedures: the ratio of detectable to non-detectable T-levels across stages was independent ($\chi^2 = 2.9$, d.f. = 1, $p = 0.09$). The fact that maximal T-levels
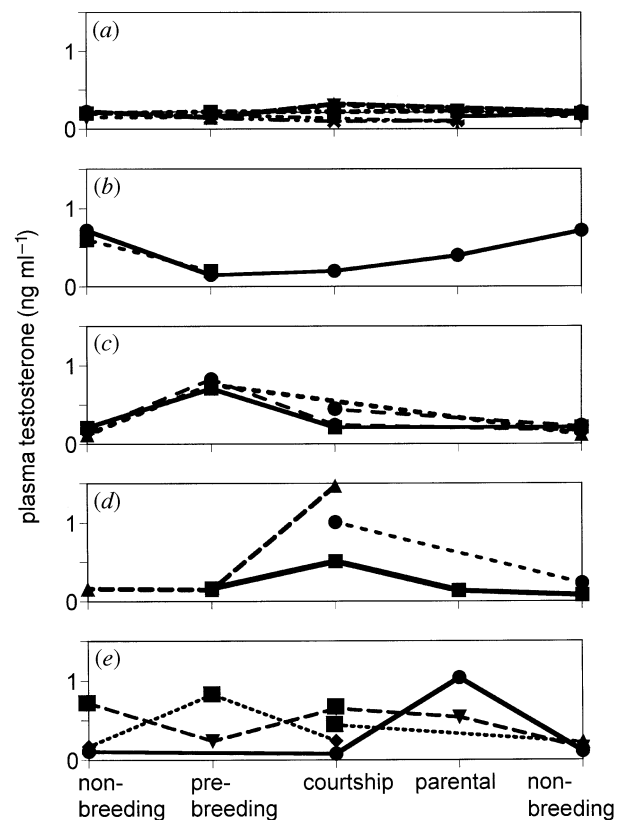


Figure 2. Plasma levels of T during four life-history stages of the annual reproductive cycle of male spotted antbirds. Each point represents one T-measurement; repeated measurements of individual birds are connected by lines. Maximum T-levels were only found during times of social challenges (intrusion of fewer than two stranger males). The different panels depict differences in the time of social instability experienced by those males: (*a*) six males experienced social stability during their entire annual cycle; (*b–e*) males were caught in socially unstable situations either during the non-breeding season (*b*), the pre-breeding season (*c*), the courtship phase (*d*), or the parental phase (*e*). Two males (squares) were caught twice during times of social challenges (*e*).

tended to be higher during the breeding season (see also Wikelski *et al.* 1999) may reflect a weak positive correlation of T-levels with gonadal volumes, the latter being elevated during the courtship phase (linear regression: T-value = 0.19 (±0.04) + 0.02 (±0.006) × gonad volume, $F_{1,94} = 17$, $p = 0.001$, $r^2 = 0.15$).

Interestingly, plasma T-levels were significantly higher when individual birds were socially challenged than when they were not challenged (Wilcoxon matched-pairs test, $n = 12$, $\mathcal{Z} = -3.06$, $p = 0.002$; figure 1*b*; again, only the maximum measured T-level of each male during times of social stability was used to avoid pseudoreplication). If this difference was due to the fact that social instability only occurred during the courtship phase and T-levels were elevated during the courtship phase, we would expect higher T-levels in challenged individuals during the courtship phase as compared to challenged individuals during any other phase of the year. This hypothesis was not supported (Mann–Whitney *U*-test, $\mathcal{Z}_{4,8} = -0.9$, $p = 0.34$; figure 1*c*). The power of this test was $r = 0.14$.

To further evaluate social influence on T-levels, we plotted individual annual T-profiles for males that

experienced social challenges at different times of their annual cycle. Six individual males had low (near baseline) T-values throughout the year (figure 2*a*). In other individuals, T-levels could be elevated at any time of the year coinciding with instances of social instability. Two males showed high T-levels during the non-breeding season at times when their gonads were entirely regressed (figure 2*b*). Three males experienced T-peaks when their gonads were still at half maximal size (figure 2*c*). Another two males had elevated T-levels during the life-history stage when one would expect elevated T-levels in temperate-zone birds, i.e. during the courtship phase (figure 2*d*). Another three males had high T-levels during the parental phase (figure 2*e*).

Next, we tested experimentally whether a simulated territorial intrusion (broadcast conspecific song) can increase plasma T-levels in spotted antbirds. We assume that the overall probability of capture was independent of plasma T because we did not find a significant relationship between capture time and T-levels (linear regression: $y = 28.3 \, (\pm 10.8) + 34.4 \, (\pm 27.2)x$, $F_{1,74} = 2.9$, $p = 0.06$, $r^2 = 0.07$). However, the duration of playback ($B$) in each territory correlated positively with the subsequently measured T-levels if we excluded socially unstable territories (open circles in figure 3; quadratic regression: T-value $= 0.21$ $(\pm 0.04) - 0.002$ $(\pm 0.001) \times B + 0.000\,045$ $(\pm 0.000\,01)$ $\times B2$, $F_{2,65} = 35$, $p < 0.001$, $r^2 = 0.52$). The quadratic model fitted significantly better than a linear one which indicated that only very long playback durations (beyond about 120 min) induced elevated T-levels. The long playback times were distributed over all seasons. It is also important to note that once attracted to playbacks, males stayed in the vicinity of the playback. There was no correlation between the duration of a playback and the number of males caught in a territory (linear regression: $p > 0.05$), indicating that we did not attract more males with longer playback times. There was also no difference in playback times between different seasons (ANOVA for four seasons: $F_{4,65} = 1.3$, $p = 0.65$), indicating that individuals were equally aggressive toward presumed territorial intruders during all seasons.

## 4. DISCUSSION

The neotropical spotted antbird breeds seasonally and all individuals regress their gonads entirely during the non-breeding season (Wikelski *et al.* 1999*b*). Despite this seasonal reproduction, plasma T-levels could be low (baseline) throughout the year. Nevertheless, both members of a pair always reacted aggressively towards playback and against territorial intruders (see also Willis 1972). Thus, despite low plasma T-levels, birds were territorial and aggressive year-round.

It is important to note that the normal range of plasma T-levels conveys biological functions in spotted antbirds. Both T-treatment and pharmacological blockage of T-action (for red-winged blackbirds, *Agelaius phoeniceus*, see Searcy & Wingfield (1980); Walters & Harding 1988) were effective in changing aggression, song and hopping activity (in spotted antbirds, Wikelski *et al.* (1999*a*); Hau *et al.* 1999*b*). This is consistent with the finding that T had important functions in other tropical birds and, for example, accumulated in the song
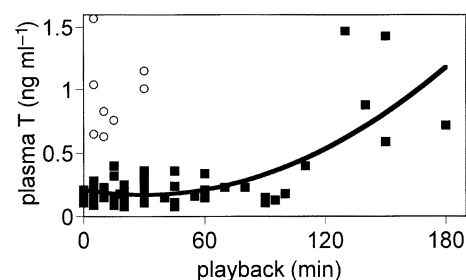


Figure 3. The plasma T-levels of males increased with the duration of playback, but only after very long ( > 120 min) playback times. The open circles were not included in the analysis because those were males caught in unstable social situations (see figure 1). Note that plasma T-levels after playback are similar to those during natural challenges.

nuclei of neotropical bay wrens (Brenowitz & Arnold 1985).

The independence of T from reproductive contexts allowed us to investigate social factors that might be responsible for eliciting physiological (endocrinological) responses. Three lines of evidence showed that plasma T-levels were enhanced by social processes. First, peak T-levels coincided with conspecific interactions (figure 1; see also Dufty & Wingfield 1985). Second, simulated territorial intrusions (conspecific playback; e.g. Wingfield 1985) enhanced T-levels at any time of year. This result differs from autumn territoriality in temperate zone birds, which do not secrete T after simulated territorial intrusion (playback plus decoy; Wingfield 1994*b*). It is so far unclear if the two-hour delay in an increase in T after playback was due to the lack of a decoy in our experiment or represents a genuinely slow physiological response. These possibilities will be subject to experimental tests.

Increased plasma T-levels after social stimulation have not been reported before for tropical birds. This is not surprising because most other studies investigated changes in the hormone levels of tropical birds only on the population level, i.e. not repeatedly in individual birds. It is thus conceivable that the variation in T-levels in tropical savannah birds reflects individual increases in T during social challenges (also discussed by Dittami & Gwinner 1990; for related phenomena in house sparrows, see Hegner & Wingfield (1986), and in lizards, see Knapp & Moore (1996)). Contrary to our findings in spotted antbirds, there are no changes in T after social challenges in a tropical bird that lives in family groups (white-browed sparrow weaver, *Plocepasser mahali*). Thus, social birds that often experience social challenges may avoid the costs of having high T-levels altogether (Wingfield *et al.* 1992; Wingfield & Lewis 1993), or have to suppress T-levels to allow for social life.

The most puzzling difference between spotted antbirds and most temperate-zone birds is that T-levels could increase during any time of the year, even when they were at the baseline levels. Temperate-zone birds can typically only increase their T to peak levels when the gonads are large and thus when T is already elevated to its breeding level (Wingfield *et al.* 1997). An increase in T to peak levels from baseline levels is unprecedented. Its proximate regulation is under investigation.

Our data on spotted antbirds confirm the challenge hypothesis in situations where territoriality and reproduction

are both coincident and separated in time. Our data also reconfirm that the physiological role of plasma T is not to influence aggression *per se* (see also Caldwell *et al.* 1984), but rather to raise the defeat threshold, for example, during fights. This, on the other hand, increases the persistence up to a point where continued fighting is detrimental to an animal. The fact that individuals with high T-levels give up too late may be the explanation for how the costs of T are physiologically mediated.

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